

## RESEARCH ARTICLE

# Three new species of *Urocleidoides* (Monogenoidea: Dactylogyridae) parasitizing characiforms (Actinopterygii: Characiformes) in Tocantins River, states of Tocantins and Maranhão, and new record for *U. triangulus* in Guandu River, state of Rio de Janeiro, Brazil

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**ABSTRACT.** Three new species of *Urocleidoides* Mizelle & Price, 1964 are described from the gills of characiform fishes in the Tocantins River and its tributaries. *Urocleidoides boulengerellae* sp. nov. is described from *Boulengerella cuvieri* (Spix & Agassiz, 1829) and differs from all its congeners by the dorsal bar with a long posteromedial projection; male copulatory organ with 2–3 counterclockwise rings and a base with a flange; an accessory piece comprising a robust Y-shaped unit and a sheath-like unit; and a highly sclerotized vaginal canal. *Urocleidoides paratriangulus* sp. nov., described from *Psectrogaster amazonica* Eigenmann & Eigenmann, 1889, *Cyphocharax gouldingi* Vari, 1992, *Caenotropus labyrinthicus* (Kner, 1858) and *Mylesinus paucisquamatus* Jégu & Santos, 1988, is most similar to *Urocleidoides triangulus* (Suriano, 1981) Rossin & Timi, 2016 based on the shape of the anchors and bars but differs from *U. triangulus* in the morphology of the projection of the dorsal bar, the number of rings of male copulatory organ, and by the smaller size of members of hook pairs 1 and 5 compared with those of the remaining pairs. *Urocleidoides tocaninensis* sp. nov. is easily distinguished from all other species of the genus by the morphology of the vagina, which present a vaginal vestibule with a membranous cap. *Urocleidoides triangulus* is reported from its type host in the Guandu River, state of Rio de Janeiro. The present study increases the number of *Urocleidoides* species to 37 recognized species that fit all the generic characters.

**KEY WORDS.** Ectoparasites, fishes, morphology, Neotropical Region, Platyhelminthes, taxonomy.

## INTRODUCTION

*Urocleidoides* was proposed by Mizelle and Price (1964) for a new species, *Urocleidoides reticulatus* Mizelle & Price, 1964 from the gills of *Poecilia reticulata* Peters (Poeciliidae), which was collected from Capitol Aquarium, Sacramento, California, USA. A few years later, 12 species were described within this genus, from the gills of fish belonging to the orders Characiformes, Cyprinodontiformes, Gymnotiformes and Siluriformes (Mizelle et al. 1968, Mizelle and Kritsky 1969, Kritsky and Leiby 1972). Subsequently, Molnar et al. (1974) described eight new species

from Trinidad and Tobago and transferred to this genus two species from *Cleidodiscus* Mueller, 1934, based on the emended diagnosis provided by Mizelle et al. (1968), which expanded the bounds of *Urocleidoides*. Kritsky et al. (1986) revised the generic diagnosis, described new species and reviewed the material belonging to known species. These authors provided redescrptions and restricted the genus to the species that presented a sinistral vaginal sclerite. Twenty-three species were thus considered incertae sedis, which reduced the number of species in the genus to five. Currently, the genus comprises 34 recognized species described in the Neotropical Region (Argentina, Brazil,

Colombia, El Salvador, Panama, Peru, Trinidad and Tobago and Mexico), that are parasites of Characiformes, Gymnotiformes and Cyprinodontiformes (Kritsky and Thatcher 1974, Molnar et al. 1974, Suriano 1997, Kritsky et al. 1986, Mendoza-Franco et al. 2007, 2015, Mendoza-Franco and Reina 2008, Rosim et al. 2011, Moreira et al. 2015, Rosin and Timi 2016, Ferreira et al. 2017, Oliveira et al. 2020, Zago et al. 2020). Although species of *Urocleidoides* have a wide distribution in the tropics, there are still few studies documenting the diversity of Monogenoidea in the Neotropical Region, considering the great biodiversity of host species. Among the species that were considered incertae sedis, eight remain uncertain and 16 have been allocated to other genera (Kritsky et al. 1986, 1989, 2000, Jogunoori et al. 2004, Mendoza-Franco et al. 2009, Yamada et al. 2015, Acosta et al. 2019).

During studies on the helminth fauna of fish in the Tocantins River, characiform fish, including *Boulengerella cuvieri* (Spix & Agassiz, 1829) (Ctenoluciidae), *Cyphocharax gouldingi* Vari, 1992 (Curimatidae), *Psectrogaster amazonica* Eigenmann & Eigenmann, 1889 (Curimatidae), *Mylesinus paucisquamatus* Jégu & Santos, 1988 (Serrasalminae) and *Caenotropus labyrinthicus* (Kner, 1858) (Chilodontidae) were examined. Characiformes is the one of the largest orders of freshwater fish, with at least 2300 valid species distributed in 520 genera (Nelson et al. 2016). These fish harbor a great diversity of helminths. However, no monogenoids have previously been described or reported from *B. cuvieri*, *P. amazonica*, *C. labyrinthicus* or *C. gouldingi*, while *Notozothecium bethae* Kritsky, Boeger & Jégu, 1996 was reported from *M. paucisquamatus* collected in the Tocantins River by Kritsky et al. (1996). In the current paper, three new species of *Urocleidoides* are described and a new distributional record for *Urocleidoides triangulus* (Suriano, 1981) Rosin & Timi, 2016 is presented.

## MATERIAL AND METHODS

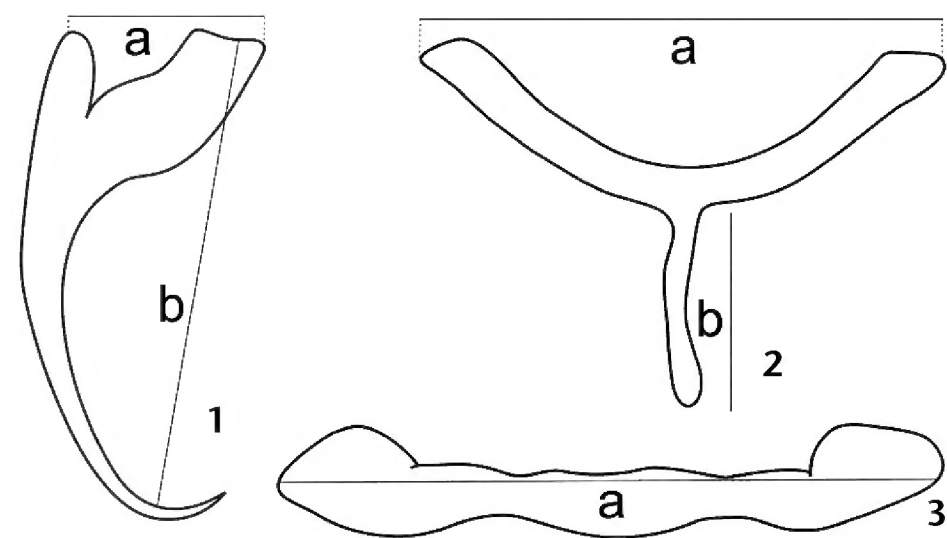
During expedition carried out in 2010 from the middle part of the Tocantins River, in the states of Maranhão and Tocantins, were collected 32 samples of *B. cuvieri* (14.5–40.8 cm in standard length and 35.3–551.0 g in weight), six of *C. gouldingi* (16.1–17.9 cm in standard length and 50–76 g in weight), six of *M. paucisquamatus* (12.3–15.5 cm in standard length and 65.2–158.2 g in weight) and seven of *C. labyrinthicus* (11.1–14.1 cm in standard length and 36–71 g in weight). These were identified by the ichthyologists from the Museu Nacional, Universidade Federal do Rio de Janeiro (UFRJ). These fish were caught with the aid of local fishermen and were examined for parasites immediately afterwards.

Ninety-seven samples of *P. amazonica* (12.0–16.8 cm in standard length and 43.0–100.0 g in weight) were collected from the urban zone of the municipality of Imperatriz, state of Maranhão, and in the village of Embiral, which is part of its rural zone, between March 2018 and August 2019. These were

identified by the specialists of the Laboratório de Sistemática e Ecologia de Organismos Aquáticos (LASEOA), Universidade Estadual do Maranhão (UEMA). These fish were caught in nets and immediately packed in Styrofoam boxes filled with ice and taken to the Laboratório de Anatomia, Universidade Estadual da Região Tocantina do Maranhão (UEMASUL), for material processing. The fish sample collection protocol and laboratory procedures were approved by the Research Ethics Committee of Universidade Estadual do Maranhão, under protocol number 21/2017, and the environmental collection license was obtained from the System for Authorization and Information on Biodiversity (SISBIO), under protocol number 61650–1. The gills were removed from the fish and placed in vials containing hot water (65 °C), which were then shaken. Formalin was added to reach a concentration of 5%.

Parasitological indexes were calculated as proposed by Bush et al. (1997), followed by standard deviation.

Monogenoids were picked out from the sediment and gill filaments in the laboratory with the aid of a stereoscopic microscope. Some specimens were mounted in Hoyer's medium so that the sclerotized parts could be studied and others were stained with Gomori's trichrome to study the internal organs of the parasite (Humason 1979). Measurements are presented in micrometers; range values are followed by mean and number of structures measured in parentheses. Measurements on the sclerotized structures of the haptor (bar and anchors) were made in accordance with the scheme shown in Figs 1–3. Morphometric studies were performed through ImageJ (Wayne 2010). This software is distributed by the National Institutes of Health (NIH), available at <https://imagej.nih.gov/ij/download.html>. Pictures were taken using a digital camera (Sony MPEG Movie EX DSC-S75) coupled to the microscope. The specimens studied were deposited in the 'Coleção Helmintológica do Instituto Oswaldo Cruz (CHIOC)' and in the collection of the 'Instituto Nacional de Pesquisas da Amazônia' (INPA) in Brazil.



Figures 1–3. Scheme of measurements of the sclerotized structures of the haptor and male copulatory organ (MCO) of *Urocleidoides* spp.: (1) anchor: (a) base, (b) total length; (2) ventral bar: (a) length, (b) medial projection; (3) dorsal bar: (a) length.

## TAXONOMY

Class Monogenoidea Bychowsky, 1937  
Subclass Polyonchoinea Bychowsky, 1937  
Order Dactylogyridea Bychowsky, 1937  
Dactylogyridae Bychowsky, 1933  
*Urocleidoides* Mizelle & Price, 1964  
*Urocleidoides boulengerellae* sp. nov.

Figs 4–11, 31, 32

<http://zoobank.org/3E609A35-9326-4217-83FF-37D5C33435D5>

Type host. *Boulengerella cuvieri* (Spix & Agassiz) (Ctenoluciidae).

Type locality. Tocantins River (8°22'55.9' S; 48° 07' 04.4' W), near the municipality of Tupiratins, state of Tocantins, Brazil.

Other localities. Arraias River (12°37'52.3' S; 47°08'11.2' W), close to the municipality of Babaçulândia, state of Tocantins; Tocantins River (6°32'24.53' S; 47° 27' 0.75' W), close to the municipalities of Aguiarnópolis and Estreito; at the mouth of the Itauéiras River (6°29'58.73' S; 47°25' 27.48' W), in the municipality of Estreito, state of Maranhão, Brazil.

Infestation parameters. Total number of hosts: 32; prevalence: 91%; total number of parasites: 876; mean intensity: 30.2±26.5; range of intensity: 2–113.

Deposited material. Holotype: CHIOC: 39560, paratypes: CHIOC 39561, 39562a-c, 39563a-b, 39564a-b, 39565, 39566; INPA 833, 834.

Description. Based on 137 specimens: 9 mounted in Gomori's trichrome and 128 mounted in Hoyers' medium: Body fusiform, delicate, 310–530 (363; n = 24) long, 63–140 (102; n = 27) wide. Tegument smooth. Cephalic region with 2 lateral lobes and 2 anterolateral slightly developed; four bilateral pairs of head organs. Eyespots absent; accessory chromatic granules present in cephalic area, Mouth subterminal, midventral; Pharynx muscular, spherical; esophagus short; two intestinal caeca, posteriorly confluent to gonads, lacking diverticula. Haptor sub-hexagonal, presenting ventral anchors connected by ventral bar and dorsal anchors connected by dorsal bar, 62–112 (94; n = 13) wide (Fig. 4). Ventral anchor with well-developed roots: protruding superficial root, rectangular in shape, deep root distally round, straight shaft and acute point, 26–44 (35; n = 43) long, base 13–26 (17; n = 31) (Fig. 8); dorsal anchor with developed roots, straight shaft and long point, 23–30 (28; n = 33) long, base 10–21 (12; n = 30) (Fig. 9). Ventral bar straight and robust, with anteromedian groove and slight protuberances at the end, 35–45 (39; n = 27) long (Fig. 10). Dorsal bar recurved, 32–40 (37; n = 14) long, with a long posteromedian projection, 9–15 (12; n = 24) long (Fig. 11). Seven pairs of marginal hooks present, ancyrocephaline distribution, five pairs ventral and two dorsal. Hooks similar in shape; pairs 1 and 5 slightly smaller than other pairs: each with protruded thumb, curved point, straight shank,

and very short filamentous hook loop about 1/6 shank length (Fig. 7). Pair 1, 10–22 (16; n = 15) long, pair 2, 18–30 (23; n = 24) long, pair 3, 19–38 (24; n = 37) long, pair 4, 16–36 (24; n = 38) long, pair 5, 15–25 (19; n = 9) long, pair 6, 20–33 (29; n = 23) long, pair 7, 16–27 (18; n = 10) long. Copulatory complex comprising male copulatory organ (MCO) and non-articulated accessory piece. MCO with 2–3 counterclockwise rings, base with flange, 61–131 (95; n = 17). Accessory piece comprising a robust Y-shaped unit and a sheath-like unit (Figs 6, 31). Testes dorsal to germarium; seminal vesicle a distal dilation of vas deferens; single prostatic reservoir present. Germarium elongated (Fig. 4). Vaginal canal a highly sclerotized tube, which coils around vaginal sclerite, connected with seminal receptacle, located anteriorly to germarium; vaginal opening ventrolateral, sinistral; vaginal sclerite present, robust, sinistral, composed of straight rod distally hooked, with a short subterminal projection, 38–50 (41; n = 11) (Figs 5, 32). Eggs, Mehlis' glands and ootype not observed. Vitellaria present, distributed throughout the body, except in area of reproductive organs (Fig. 4).

Etymology. the specific name refers to the scientific name of the host.

Remarks. The new species is allocated in *Urocleidoides* by the presence of vaginal sclerite, MCO with counterclockwise rings and pairs 1 and 5 reduced in size. *Urocleidoides boulengerellae* sp. nov. can be differentiated from all other species of the genus mainly by the morphology of the copulatory complex and through the highly sclerotized vaginal canal. *Urocleidoides boulengerellae* sp. nov. is most similar to *Urocleidoides neotropicalis* Mendoza-Franco & Reina, 2008 and *Urocleidoides piriatiu* Mendoza-Franco & Reina, 2008, by the long posteromedian projection in the dorsal bar. However, the new species differs from *U. neotropicalis* by the morphology of the male copulatory organ (2–3 rings in *U. boulengerellae* sp. nov. and a coil of about 5½ counterclockwise rings in *U. neotropicalis*), while it differs from *U. piriatiu* by the shape of the subunits of the accessory piece (an Y-shaped, robust and a sheath-like unit in *U. boulengerellae* sp. nov. and dextral subunit terminally acute; sinistral subunit bottle-shaped in *U. piriatiu*).

### *Urocleidoides paratriangulus* sp. nov.

Figs 12–21, 33, 34

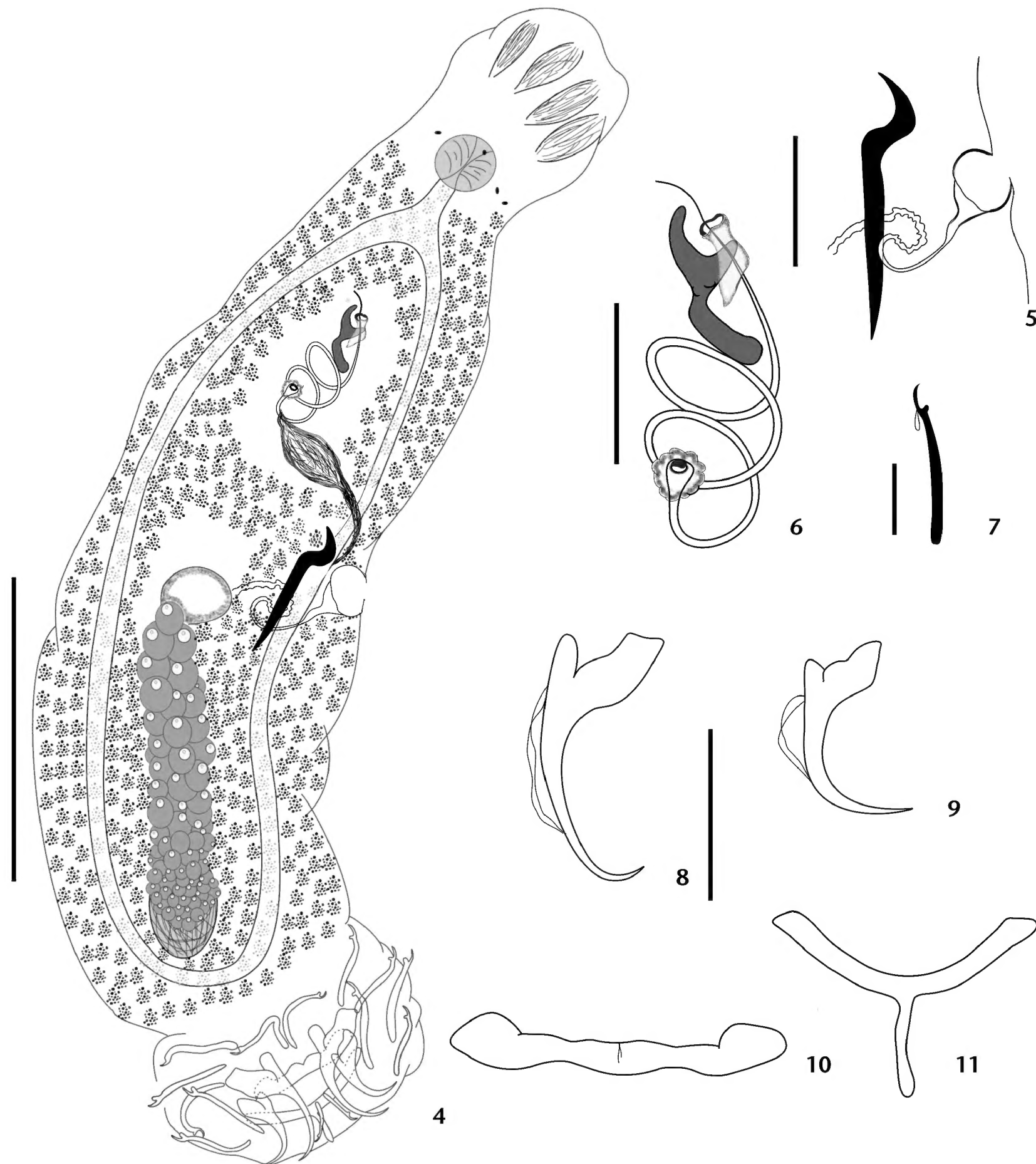
<http://zoobank.org/3C18E487-2368-45B7-BB00-2A7F03AAE9AA>

Type host. *Psectrogaster amazonica* Eigenmann & Eigenmann (Curimatidae).

Type locality. Tocantins River (5°27'50'S; 47°33'48'W), close to the municipality of Embiral, state of Maranhão.

Other hosts and localities. *Psectrogaster amazonica*: Tocantins River (5°32'08.6'S; 47°29'41.1'W), close to the urban perimeter of Imperatriz, state of Maranhão. *Cyphocharax gouldingi* (Curimatidae): Tocantins River (6°32'24.53'S; 47°27'0.75'W), close to the municipalities of Aguiarnópolis and Estreito; at the mouth of the Itauéiras River (6°29'58.73'S; 47°25'27.48'W), in the municipality

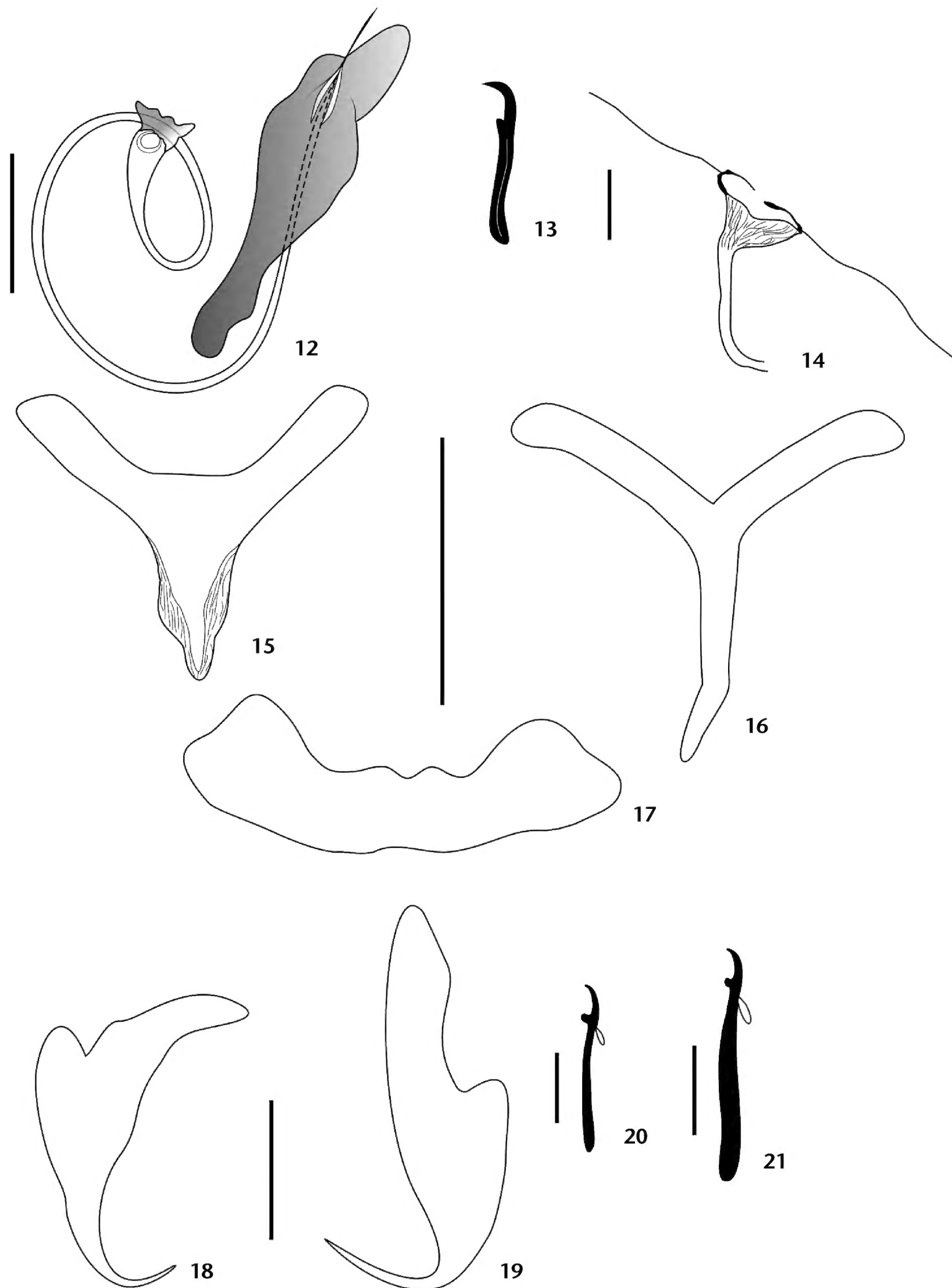




Figures 4–11. *Urocleidoides boulengerellae* sp. nov. from *Boulengerella cuvieri* from Tocantins River: (4) total, ventral view; (5) vaginal sclerite and vagina; (6) copulatory complex, ventral view; (7) hook; (8) ventral anchor; (9) dorsal anchor; (10) ventral bar; (11) dorsal bar. Scale bars: 4 = 100  $\mu$ m, 5 = 20  $\mu$ m, 6 = 30  $\mu$ m, 7 = 10  $\mu$ m, 8–11 = 20  $\mu$ m.

of Estreito, state of Maranhão. *Caenotropus labyrinthicus* (Chilodontidae): Arraias River (12°37'52.3'S; 47°08'11.2'W), close to the municipality of Babaçulândia, state of Tocantins; Tocantins River (6°32'24.53'S; 47°27'0.75'W), close to the municipalities of Aguiarnópolis and Estreito; at the mouth of the Itaueiras River (6°29'58.73'S; 47°25'27.48'W), municipality of Estreito, state of Maranhão; João Aires River (7°51'10.6'S; 47°55'57.3'W), close to

the municipality of Palmeirantes, state of Tocantins; Farinha River (6°50'30.5'S; 47°30'05.8'W), close to the municipality of Estreito, state of Maranhão, Brazil. *Mylesinus paucisquamatus* Jégu & Santos (Serrasalmidae): Arraias River (12°37'52.3'S; 47°08'11.2'W), close to the municipality of Babaçulândia, state of Tocantins; Farinha River (6°50'30.5'S; 47°30'05.8'W), close to the municipality of Estreito, state of Maranhão, Brazil.



Figures 12–21. *Urocleidoides paratriangulus* sp. nov. from *Psectogaster amazonica* from Tocantins River: (12) copulatory complex, ventral view; (13) vaginal sclerite; (14) vagina; (15, 16) dorsal bar; (17) ventral bar; (18) ventral anchor; (19) dorsal anchor, (20) hook pairs 1, 5; (21) hook pairs 2–4, 6, 7. Scale bars: 12 = 10 µm, 13–14 = 10 µm, 15–17 = 20 µm, 18–19 = 20 µm, 20 = 5 µm, 21 = 10 µm.

Infestation parameters. *Psectrogaster amazonica*: total number of hosts: 97; prevalence: 11.3%; total number of parasites: 37, mean intensity:  $3.1 \pm 2.5$ , range of infection: 1–9; *Caenotropus labyrinthicus*: total number of hosts: 8; number of infected hosts: 3; total number of parasites: 4; *Cyphocharax gouldingi*: total number of hosts: 8; number of infected hosts: 1; total number of parasites: 2; *Mylesinus paucisquamatus*: total number of hosts: 7; number of infected hosts: 1; total number of parasites: 10.

Deposited material. Holotype: *Psectrogaster amazonica*: CHIOC: 39567a; paratypes CHIOC 39567b, 39571, 39572, 39573, 39574, INPA 836, 837. *Cyphocharax gouldingi*: CHIOC: 39568a-b. *Caenotropus labyrinthicus*: CHIOC: 39570, *Mylesinus paucisquamatus*: CHIOC: 39569a-c, INPA 835.

Description. Based on 51 specimens mounted in Hoyers' medium: Body fusiform, robust 195–402 (290; n = 15) long by 65–132 (103; n = 15) wide. Cephalic region with cephalic lobes poorly developed; four bilateral pairs of head organs. Eyespots absent; accessory chromatic granules dispersed in the cephalic region. Mouth subterminal, midventral; pharynx spherical, esophagus short; two intestinal caeca, confluent posteriorly to testes. Haptor sub-hexagonal, 60–105 (83; n = 15) wide (Fig. 34). Ventral anchor with superficial root elongated, round deep root knob-like, straight shaft and short point, 60–105 (83; n = 15) long, base 13–31 (22; n = 38) (Fig. 18). Dorsal anchor with well-developed elongated superficial root and inconspicuous deep root, short shaft and long point, 26–50 (36; n = 27) in length and base 15–30 (22; n = 28) (Fig. 19). Ventral bar straight, with an anteromedian groove and rounded extremities, anteriorly directed, 24–45 (33; n = 15) long (Fig. 17). Dorsal bar V-shaped, postero-median projection 19–38 (24; n = 12) long, with two variations: a slender or a more robust projection with lateral allae, 33–47 (40; n = 11) long (Figs 15, 16). Seven pairs of robust marginal hooks, five ventral and two dorsal, similar in shape, pairs 1 and 5 reduced in size, presenting curved point, dilated shank, composed of one subunit, protruded thumb, filamentous hook loop about 1/6 shank length (Figs 20, 21), pair 1, 7–20 (13; n = 30) long; pair 2, 18–51 (35; n = 23) long; pair 3, 17–37 (28; n = 20) long; pair 4, 15–27 (21; n = 19) long; pair 5, 9–14 (11; n = 17) long; pair 6, 14–42 (29; n = 22) long; pair 7, 6–43 (21; n = 21) long. Copulatory complex consists of male copulatory organ (MCO) and accessory piece, which serve as guide to MCO. Male copulatory organ about two counterclockwise rings, the proximal ring has a smaller diameter than the distal one, 54–100 (75; n = 18) long. Accessory piece not connected to MCO, situated at distal portion of the organ, consisting of a shaft with medial portion enlarged, with distal groove supporting MCO, 21–38 (28; n = 10) long (Figs 12, 33). Vagina ventral, funnel-shaped, consisting of a sclerotized tube, opening dorsally (Fig. 14). Vaginal sclerite composed of a long rod, longitudinally grooved, distal extremity hooked and subterminal short projection, 18–32 (23; n = 13) long (Fig. 13). Eggs, Mehlis' glands and ootype not observed. Vitellaria distributed throughout the body, absent in the region of reproductive organs.

Etymology. The specific name refers to the similarity with *Urocleidoides triangulus*.

Remarks. *Urocleidoides paratriangulus* sp. nov. is similar to *U. triangulus* considering the shape of the anchors and bars. The new species differs from *U. triangulus* by the projection of the dorsal bar (prominent postero-median process in *U. triangulus* and slender or a more robust projection with lateral allae in the new species); the number of rings of MCO (2.5 to 3 in *U. triangulus* and about two in the new species); and by the reduced size of hooks (pairs 1, 5 and 7 reduced in size in *U. triangulus* and 1 and 5 only in *U. paratriangulus* sp. nov.). Only five species of *Urocleidoides* possess a medial projection on the posterior margin of the dorsal bar: *Urocleidoides curimatae* Molnar, Henek & Fernando, 1974; *U. neotropicalis*; *U. piria-tiu*; *Urocleidoide tenuis* Zago, Yamada, Yamada, Franceschini, Bongiovani & Silva, 2020; and *U. boulengerellae* sp. nov. The medial projection is slenderer than the bar arms in the five species, whereas in *U. triangulus* and in some specimens of *U. paratriangulus* sp. nov., the projection is thicker than the arms. *Urocleidoides paratriangulus* is similar to *U. curimatae* and *U. tenuis* also in terms of the triangular shape of the ventral anchor, but it can be differentiated by the number of coils and the length of the MCO (3 coils in *U. paratriangulus* sp. nov. vs 1.5 coils in *U. curimatae* and 7.5 in *U. tenuis*), as well as through the morphology of the accessory piece (curved shaft in *U. paratriangulus* sp. nov., straight in *U. curimatae* and pincer-shaped in *U. tenuis*). During a study carried out in the Guandu River by the laboratory team, specimens of *U. triangulus* were found parasitizing *Cyphocharax gilbert* (Quoy & Gaimard, 1824). The morphometry of the specimens studied herein were used to make comparisons with those of *U. paratriangulus* sp. nov. This was the first record in this host in Brazil.

### *Urocleidoides tocantinensis* sp. nov.

Figs 22–30, 35, 36

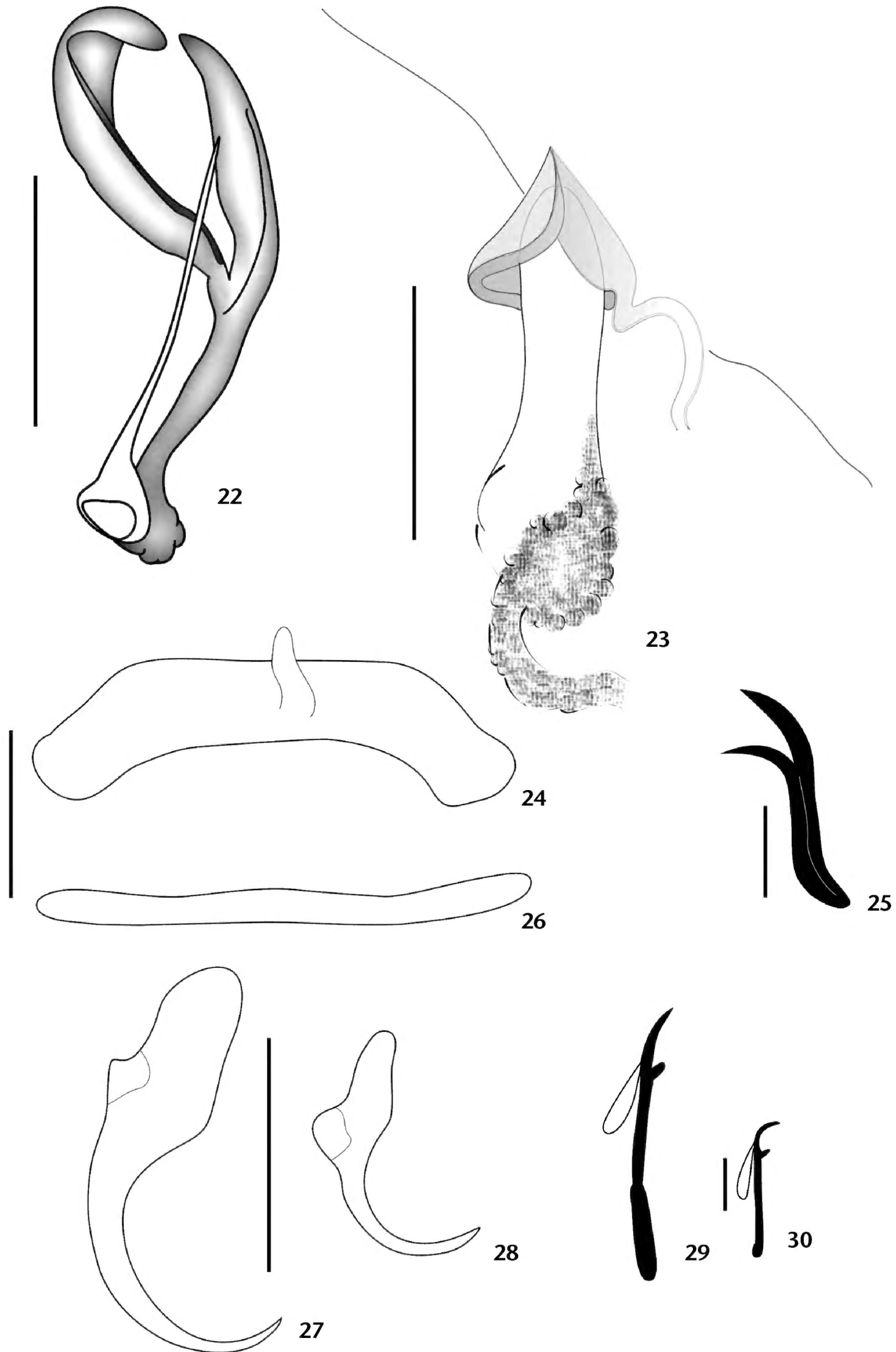
<http://zoobank.org/96A69E56-0836-4AC2-85A3-3C486B873C33>

Type host. *Psectrogaster amazonica* Eigenmann & Eigenmann (Curimatidae).

Type locality. Tocantins River (5°27'50" S; 47°33'48" W), close to the municipality of Embiral, state of Maranhão.

Other hosts and localities. *Psectrogaster amazonica*: Tocantins River (5°32'08.6" S; 47°29'41.1" W), close to the urban perimeter of Imperatriz, state of Maranhão. *Mylesinus paucisquamatus* (Serrasalminidae): Arraias River (12°37' 52.3" S; 47°08'11.2" W), close to the municipality of Babaçulândia, state of Tocantins; Farinha River (6°50'30.5" S; 47°30'05.8" W), close to the municipality of municipality of Estreito, state of Maranhão, Brazil.

Infestation parameters. *Psectrogaster amazonica*: total number of hosts: 97; prevalence: 9.3%; total number of parasites: 11; mean intensity:  $1.22 \pm 0.44$ ; range of infection: 1–2; *Mylesinus paucisquamatus*: total number of hosts: 7; number of infected hosts: 2; total number of parasites: 12.



Figures 22–30. *Urocleidoides tocantinensis* sp. nov. from *Psectogaster amazonica* from Tocantins River: (22) copulatory complex, ventral view; (23) vagina; (24) ventral bar; (25) vaginal sclerite; (26) dorsal bar; (27) ventral anchor; (28) dorsal anchor; (29) hook pairs 2–4, 6, 7; (30) hook pairs 1, 5. Scale bars: 22, 23, 27, 28 = 20 µm, 24–26, 29 = 10 µm, 30 = 5 µm.





Figures 31–36. Light photomicrographs of *Urocleidoides* spp.: (31, 32) *Urocleidoides boulengerellae*: (31) copulatory complex; (32) vaginal sclerite; (33, 34) *Urocleidoides paratriangulus*: (33) copulatory complex; (34) haptor; (35, 36) *Urocleidoides tocantinensis*: (35) copulatory complex; (36) vagina. Scale bars: 31–33, 35–36 = 20  $\mu$ m, 34 = 40  $\mu$ m.

Deposited material. *Psectrogaster amazonica*: Holotype CHIOC 39575; paratypes 39577, 39578; INPA 838; *Mylesinus paucisquamatus*: CHIOC 39576a-b, INPA 839.

Description. Based on 15 specimens: 2 mounted in Gomori's trichrome and 13 mounted in Hoyer's medium. Body

fusiform, robust, 200–317 (278; n = 8) long by 68–103 (89; n = 9). Cephalic lobes poorly developed; two pairs of eyespots, posterior pair larger than anterior; accessory chromatic granules dispersed in the cephalic region. Pharynx spherical; esophagus short; two intestinal caeca, posteriorly confluent to gonads,



lacking diverticula. Haptor sub-hexagonal, 62–86 (68;  $n = 12$ ) wide. Ventral anchor with developed superficial root and inconspicuous deep root, evenly curved shaft and point, 21–44 (33;  $n = 22$ ) long and base 13–20 (17;  $n = 22$ ) (Fig. 27). Dorsal anchor, smaller than ventral, with well-defined and long superficial root, shorter deep root, evenly curved shaft and robust point, passing from the level of tip of superficial root, 15–29 (22;  $n = 18$ ) long and base 10–19 (13;  $n = 16$ ) (Fig. 28). Ventral bar straight, with extremities directed toward posterior extremity, with a median projection arising from the middle portion of the bar, 23–34 (30;  $n = 9$ ) long (Fig. 24). Dorsal bar straight, 25–41 (30;  $n = 6$ ) long (Fig. 26). Marginal hooks present, dissimilar in shape: pairs 1 and 5 with straight shank, erected thumb and curved point; filamentous hook (FH) loop about 1/3 of shank length (Fig. 30), pairs 2–4, 6, 7 with dilated shank composed of two subunits, erected thumb and point, FH loop about 1/3 shank length (Fig. 29). Pair 1, 9–16 (13;  $n = 15$ ); pair 2, 15–27 (19;  $n = 19$ ); pair 3, 14–22 (18;  $n = 18$ ); pair 4, 15–27 (17;  $n = 17$ ); pair 5, 10–15 (13;  $n = 14$ ), pair 6, 15–25 (20;  $n = 6$ ); pair 7, 13–25 (20;  $n = 14$ ). Copulatory complex composed of male copulatory organ (MCO), which is a straight tube, 23–40 (33;  $n = 14$ ) long and by bifurcated accessory piece, serving as guide to MCO; accessory piece 41–70 (53;  $n = 15$ ) long (Figs 22, 35). Gonads overlapping. Vaginal pore sinistral, vaginal vestibule, with a membranous cap at the aperture, tubular, expanded, 28–41 (35;  $n = 15$ ), giving rise to vaginal canal (Figs 23, 36). Vaginal sclerite composed of a grooved rod, distal hook and a long subterminal projection, 22–35 (27;  $n = 11$ ) (Fig. 25). Eggs, Mehlis' glands and ootype not observed. Vitelline follicles distributed throughout the body, except in the region of reproductive organs.

**Etymology.** The specific name refers to the locality, Tocantins River.

**Remarks.** *Urocleidoides tocantinensis* sp. nov. resembles *Urocleidoides falxus* Zago, Yamada, Yamada, Franceschini, Bongiovani & Silva, 2020 by the absence of coils on the MCO and *Urocleidoides surianoae* Rosin & Timi, 2016 in the shape of accessory piece, but differs in the morphology of the MCO (MCO reverse J-shaped, with expanded proximal end in *U. surianoae* and a straight tube in the new species). The new species differs from all other species of the genus by the morphology of the vagina, which present a vaginal vestibule with a membranous cap.

## DISCUSSION

To date, 34 species of *Urocleidoides* are known, described from 44 different host species. *Urocleidoides* is mainly characterized by the presence of a sclerotized structure located in the median region of the body, named vaginal sclerite. At present, species of *Urocleidoides* are found in fishes from Argentina, Brazil, Colombia, El Salvador, Guatemala, Mexico, Panama and Trinidad and Tobago. Although there is not enough information to reconstruct the historical associations of *Urocleidoides* spp., there is plenty of evidence that their lineages can parasitize a diverse

range of host, from the fact that they are parasites of 10 different families belonging to three orders (Characiformes, Cyprinodontiformes and Gymnotiformes) (Mizelle and Price 1964, Kritsky et al. 1986, Moreira et al. 2015, Ferreira et al. 2017). A phylogenetic analysis using morphological and molecular data has suggested that Characiformes is a sister group of Gymnotiformes (Briggs 2005). However, the parasite data is still at too early a stage to be able to say whether divergence between these two latter fish groups in South America might also represent the earliest phylogenetic split of *Urocleidoides* in the tropics (Saitoh et al. 2003, Briggs 2005, Mendoza-Franco and Reina 2008).

Species of *Urocleidoides* have also been found in aquarium fishes, generally those collected from the native habitats in the Neotropical Region, such as *U. reticulatus* from *Poecilia reticulata* and *Urocleidoides vaginoclastrum* Jogunoori, Kritsky & Venkatanarasaiah, 2004, from *Xiphophorus hellerii* Heckel, 1848 (type host). Hosts of these two species have been introduced into aquaria and ponds in Israel, Czech Republic, India, California (USA), and central Mexico (see Kritsky et al. 1986, Jogunoori et al. 2004, Mendoza-Palmero & Aguilar-Aguilar 2008).

*Urocleidoides boulengerellae* sp. nov. was found only in one host species, *B. cuvieri*, with a high prevalence, mean intensity, and abundance. In Brazil, the distribution of *B. cuvieri* is restricted to the Amazon and Tocantins-Araguaia basins, in the states of Pará, Amapá, and north of Mato Grosso. The new species was not found in any other host examined in the present study, and thus can be considered to be a specialist, as the majority species of the genus. On the contrary, *U. tocantinensis* sp. nov. was found in host species belonging to different families: *M. paucisquamatus* (Serrasalminidae) and *P. amazonica* (Curimatidae), likewise, *U. paratriangulus* sp. nov. was found in hosts of different families, thus demonstrating that these species are more generalist. This scenario is similar to what has been observed for a few other species of this genus, such as *Urocleidoides eremitus* Kritsky, Thatcher & Boeger, 1986 which was found parasitizing hosts belonging to Anostomidae and Erythrinidae. All of the remaining species that occur in more than one host species are restricted to a single host family.

Rosim et al. (2011) postulated that the presence of a ventral bar with enlarged extremities may be also a characteristic that can be considered to be diagnostic for *Urocleidoides*, in addition to those proposed by Kritsky et al. (1986) in the revision of the genus. Zago et al. (2020) proposed an amended diagnosis for the genus, considering the recent discovery of new *Urocleidoides* species. Among these features considered was the absence of coil in the MCO, which groups together *U. falxus* and *Urocleidoides tocantinensis* sp. nov.

*Urocleidoides triangulus* was originally described from *C. gilberti*, as *Andropira triangula* from the Chascomús lagoon, Argentina, by Suriano (1981). Suriano (1997) provided a redescription of the species and proposed a new combination, allocating the species in *Palombitrema*, as *P. triangulum*. Rosin and Timi (2016) studied the monogenoidean fauna of *Cyphocharax voga* (Hensel,

1870): they found this species and proposed a new combination, *U. triangulus*, based on having identical morphology of the copulatory complex, hooks and internal organs, specially by the presence of a vaginal sclerite. *Urocleidoides triangulus* has been reported parasitizing different species of *Cyphocharax*: *C. voga* (Rosin and Timi 2016), *C. gilbert* in Argentina (Suriano 1981, 1997), *C. modestus* (Fernández-Yépez) (Abdallah et al. 2015) and *C. nagellii* in Brazil (Steindachner) (Vieira et al. 2013, Abdallah et al. 2015). The present report provides the first record in the type host in Brazil.

The data obtained in the present study increases the number of *Urocleidoides* species to 37 and shows that further studies are necessary to clarify the morphological characteristics that limits the diagnosis of this genus.

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